

1 EGFR 37 KDA FRAGMENT AS CANCER MARKER

2

3 The present invention relates to a method of diagnosis
4 of bladder cancer or prostate cancer and to a method of
5 detecting recurrence of bladder or prostate cancer.
6 More particularly the invention relates to an
7 accessible marker.

8

9 Transitional cell carcinoma (TCC) of the bladder
10 accounts for 1% of all cancers and is the fifth most
11 common malignancy in people over the age of sixty in
12 industrialised parts of the world (Russell et al.,
13 1988; Gleave et al., 1993). Eighty percent of all
14 bladder TCC is superficial at presentation; the
15 remaining 20% is muscle invasive and 50% of patients in
16 this category die despite treatment (Simoneau and
17 Jones, 1994). Of those patients initially presenting
18 with superficial tumours, 50 to 70% have recurrences
19 within two years. These recurrences are usually
20 superficial, although 10 to 20% progress to the muscle
21 invasive form (Parmer et al., 1989; Fradet, 1992;
22 Harland, 1994).

23

24 The high frequency of recurrent TCCB and the increase
25 in disease status in a proportion of patients means

1 that lifetime follow-up using cystoscopy and urinary
2 cytology is essential. The standard procedure is an
3 initial check cystoscopy three months after disease
4 presentation; if this is clear cystoscopy should then
5 be carried out every six months, for one to two years
6 and then annually thereafter with a flexible
7 cystoscope. At present the recurrence rate of TCCB
8 means that annual lifetime cystoscopies should be
9 carried out for all stabilised patients.

10

11 Cystoscopy involves insertion of a cystoscope into the
12 bladder via the urethra to allow visualisation of the
13 tumour using fibre optics. It confirms clinically and
14 pathologically the presence of tumour within the
15 bladder and allows a morphological description (Hossan
16 and Striegal 1993). However it has the disadvantages
17 of being an invasive, uncomfortable procedure. The
18 frequent recurrences of TCCB mean that patients must
19 undergo lifetime follow-up using cystoscopy; this
20 results in the further disadvantage of a large
21 expenditure by the health service.

22

23 Urine cytology is used for the detection of recurrent
24 bladder TCC and although it offers the advantages of
25 being a non-invasive, inexpensive, easily accessible
26 procedure (Zein and Milad, 1991), it has a poor
27 sensitivity, especially at lower stages and grades of
28 disease. The result is false positive and negative
29 findings with reported sensitivities ranging from 37.9%
30 (Miyanaga eta al., 1997) to 64% (Martins et al., 1997).

31

32 Numerous studies have been carried out to find the
33 ideal bladder cancer marker. However, none are
34 adequately sensitive or specific enough to fulfil a
35 diagnostic role at present. The most successful to
36 date appears to be the Bard BTA, STAT and TRAK tests

1 with overall sensitivities of 55% (Bard promotional
2 information), 72% (Leyh et al., 1997) and 88% (Bard
3 promotional information) respectively.

4

5 Bladder cancer is a frequently recurring disease;
6 patients require lifetime monitoring using cystoscopy
7 and urinary cytology. Cystoscopy is an invasive
8 technique and urinary cytology while non-invasive has a
9 low sensitivity.

10

11 It is an aim of the present invention to replace these
12 two procedures with a sensitive, non-invasive urinary
13 test which would allow detection of first presentation
14 and recurrent bladder cancer.

15

16 The invention relates to the presence of a 37KDa
17 epidermal growth factor receptor (EGFR) fragment in the
18 urine of patients with transitional cell carcinoma of
19 the bladder (TCCB) and in the urine of some patients
20 with prostate cancer.

21

22 This fragment had not previously been detected and its
23 presence permits the development of a novel and
24 inventive diagnostic test.

25

26 The 37KDa fragment can be observed in a western blot of
27 proteins from a urine sample from a patient with TCCB.

28

29 According to the present invention there is provided a
30 marker for bladder cancer, the marker comprising a
31 37KDa EGFR fragment which is detectable in urine.

32

33 The marker may also or alternatively be used as a
34 marker for prostate cancer.

35

36 The invention provides a test for the presence of a

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1 37KDa EGFR fragment in urine, the test comprising
2 detecting the 37KDa EGFR fragment with an antibody.
3 The test may comprise a western blot assay.

4
5 Alternatively the test may comprise an
6 immunochromatographic assay, an ELISA test, latex
7 agglutination or radioimmunoassay.

8
9 The invention further provides a method of diagnosing
10 bladder cancer or prostate cancer or detecting
11 recurrence of these, the method comprising the steps of
12 reacting a urine sample from an individual to be tested
13 with means to detect a 37KDa EGFR fragment and
14 analysing results.

15
16 Herein the term "diagnosing" relates to first
17 presentation diagnosis and detection of recurrence

19 In one embodiment the means to detect the 37KDa EGFR
20 fragment is an antibody.

21
22 Preferably the antibody is raised against a peptide
23 corresponding to amino acid residues 1005 to 1016 of
24 EGFR or binds to such a peptide or a peptide
25 substantially similar thereto.

27 A substantially similar peptide is 60% homologous to
28 the amino acid sequence along at least 50% of the
29 length of the 37kDa peptide.

30
31 In a particular embodiment of the invention the
32 antibody is Ab4 EGFR antibody available from Oncogene
33 Science, Inc.

34
35 The invention further provides the use of antibody Ab4
36 EGFR in a test to detect the presence of 34KDa EGFR

1 fragment in urine.

2

3 The invention also encompasses the use of specific
4 antibodies raised to the 37KDa fragment of EGFR.

5

6 In one embodiment the test is in the form of a dip _
7 stick.

8

9 The test can be used in conjunction with other
10 appropriate tests to diagnose TCCB, prostate cancer and
11 urinary infection.

12

13 **Experiment 1**

14

15 A 37KDa EGFR fragment has been detected in urine from
16 patients with bladder cancer. First morning urine
17 samples were collected from 24 TCC patients, 6 patients
18 who had bladder cancer previously but who were now
19 disease free and 13 healthy volunteers. 10mls of urine
20 from each was freeze dried and the powdered residue
21 reconstituted in Laemmli lysis buffer. After heating
22 at 110°C for 20 minutes, all samples were stored at -
23 70°C until required for analysis. Samples were then
24 probed with the Ab4 EGFR antibody (Oncogene Sciences)
25 to the internal domain of the receptor by western blot
26 analysis.

Disease Status	No	Presence of the 37KDA Fragment	Absence of the 37KDA Fragment
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Healthy	13	1	12
TCC	24	21	3
Remission (disease free)	6	4	2

27 A 37KDa fragment was detected in 88% (21/24) of TCC
28 patients, 66% (4/6) of disease free patients and 7%
29 (1/13) of healthy volunteer urine samples. There was

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1 an overall significant association between detection of
 2 the 37KDa fragment and presence of bladder cancer.
 3 Although four out of six patients who were thought to be
 4 disease free tested positively, two had frank low grade
 5 tumours and two had bladder inflammation at the time
 6 the urine sample was taken. This 37KDa fragment
 7 therefore appears to be of diagnostic importance. It
 8 has a much higher sensitivity than urinary cytology and
 9 the Bard BTA and STAT tests, and it appears to be
 10 comparable to the Bard TRAK test.

11

12 **Experiment 2**

Disease Status	Number	Presence of the 37KDA Fragment	Absence of the 37KDA Fragment	(CHI) ²
Healthy	25 (13)	1 (4%)	24 (96%)	
Urinary Infection	16 (12)	10 (62.5%)	6 (37.5%)	
Remission (disease free)	6 (2) †	0	6 (100%)	46.17*
TCC	32 (24)	28 (87.5%)	4 (12.5%)	
Prostate Cancer	10 (0)	5 (50%)	5 (50%)	

13 **Sensitivity levels for the detection of a 37KDa EGFR
 14 fragment in urine.**

15

16 * denotes significant ($p < 0.001$); † number in brackets is
 17 the number originally reported.

18

19 † This is somewhat different from Experiment 1 - the 6
 20 so called remission patients were in fact all in
 21 remission when the notes were checked.

22

23 In fact: two were in remission, BUT two had

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1 inflammation and two frank low grade tumour - and have
2 been reassigned. Four more patients who are definitely
3 in remission at the time of the test were added and
4 there are now 6 confirmed remission patients with no
5 marker.

6

7 Overall the second study has increased the number by a
8 small amount and the data is holding up well. A group
9 of prostate cancer patients has been added in since
10 males often have undiagnosed prostate cancer. This
11 could be a confounding factor as 50% are positive.
12 However there is a blood test for prostate cancer so
13 this would have to be carried out on positive patients
14 along with a check for infection.

15

16 It is possible that the 37KDa protein could be used to
17 distinguish between stage or grade in prostate cancer.
18 The biology of prostate should be clarified and then
19 collated with the patients tested. The test could be
20 used as a general screen for health in the
21 genitourinary area since it might pick up silent
22 bladder and prostate tumours and infection - a positive
23 test could lead to other tests to rule these
24 possibilities out.

25

26 **Comment on the table:**

27

28 - shows 87.5% of TCC patients tested positive for
29 the protein, whereas in contrast only 4% of the
30 healthy controls expressed this protein in urine

31

32 - those patients in disease free (in remission),
33 100% tested negative

34

35 - the urinary infection group, 62.5% of the patients
36 tested positive and 37.5% tested negative

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- 1 - - 50% of the prostate cancer patients test positive
- 2
- 3 - to date, the overall sensitivity of the 37KDa
- 4 protein is 87% and the specificity is 96%.
- 5
- 6 - statistical analysis shows that detection of the
- 7 37KDa fragment is dependent on the presence of
- 8 disease ($\chi^2=46.17$ $p<0.001$).

10 Detection of the 37kDR EGFR fragment in urine

12 From the investigations carried out on the detection of
13 the 37KDa EGFR fragment, it has been statistically
14 established that the detection of the protein is
15 dependent on disease presence. The fact that all
16 remission patients analysed, tested negative for the
17 37KDa fragment is very encouraging. To date the
18 overall sensitivity of the fragment protein is 87% and
19 the specificity is 96%. Both these figures are
20 superior to those of the BTA stat and the NMP22 tests
21 which are commercially available. The sensitivities
22 for the NMP22 and the BTA stat are 48% and 57%
23 respectively, with specificites of 70% and 68%
24 respectively (Weiner et al, 1998). However, the 37KDa
25 EGFR fragment test is not 100% sensitive or specific.
26 The test did not pick up 4 patients who had bladder
27 tumours at the time of analysis. It may therefore be
28 suggested that the 37KDa test could be used in tandem
29 with both the NMP22 and the BTA stat test to reach 100%
30 sensitivity and specificity. If 2 out of 3 of the
31 tests gave positive results for a particular patient,
32 it could be predicted that the patient had a bladder
33 tumour. However, this hypothesis needs to be
34 researched further, in order for this statement to be
35 confirmed.

1 The test of the present invention may be used alone or
2 together with any other suitable test.

3

4 Of the prostate patients analysed, 50% tested positive
5 for the 37kDa fragment. The medical records of these
6 patients will have to be researched further to confirm
7 if they also had a undetected bladder tumour at the
8 time of urine analysis. If it is found that these
9 patients did not have bladder cancer, they could be
10 ruled out by performing the prostate-specific antigen
11 (PSA) test.

12

13 From the data obtained it was also found that 57% of
14 urinary infection patients tested positive for the
15 37KDa fragment. This was to be expected, as EGFR over
16 expression has been associated with inflammation and
17 chronic irritation (Uhlman et al., 1996). The urinary
18 infection patients would have to be treated with a
19 course of antibiotics before the 37KDa test could be
20 carried out. The 37KDa fragment test has a number of
21 clinical uses. Firstly, the test could be used to
22 determine whether or not a patient requires cystoscopy.
23 This would cut down on the number of cystoscopies
24 presently carried out and would save the National
25 Health Service considerable expense. The test would
26 also be less traumatic for the patient than having
27 cystoscopy, which is an uncomfortable, time consuming
28 procedure. As males are becoming more aware of their
29 own health, the test could also be used to screen males
30 over 50 years, as this is the group most at risk from
31 bladder cancer. It is hoped that a urinary dip-stick
32 will allow quick detection of the presence of a bladder
33 tumour.

34

35 The high frequency of recurrent TCC in the bladder and
36 the progression to a more malignant phenotype in a

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1 proportion of patients means that lifetime follow-up
2 using cystoscopy and urinary cytology is essential.
3 Cystoscopy is an invasive procedure and urinary
4 cytology while non-invasive is relatively insensitive.
5 At present the Bard BTA and STAT tests are the only
6 commercially available detectors for bladder cancer.
7 Their sensitivity means that at best they will only act
8 in conjunction with cystoscopy. The Bard TRAK test
9 while more sensitive has yet to be marketed and in fact
10 the results from the present study indicate that the
11 37KDa EGFR fragment is at least comparable. Further
12 work is required to investigate the significance of
13 this fragment in the detection of first presentation
14 and recurrent bladder TCC and to determine whether
15 making it into a quantitative test will offer some
16 insight into prognosis. Appropriate applications are
17 detailed below.

18

19 The 37KDa EGFR fragment may be used as a detector for
20 first presentation bladder and recurrent bladder TCC.
21 Detection of the 37KDa EGFR fragment may be carried out
22 by other methods of investigation as well as western
23 blot analysis. These methods may include
24 immunochromatography, ELISA, latex agglutination or
25 radioimmunoassay. There is currently available a one-
26 step immunochromatographic assay which qualitatively
27 detects bladder tumour antigen in urine in five
28 minutes. Detection of the 37KDa EGFR fragment may be
29 detected by a similar method. Patient urine would be
30 added to the small chamber where it mixes with a
31 colloidal gold-conjugated antibody. If the 37KDa
32 fragment is present, a 37KDa fragment conjugate complex
33 would form. The reaction mixture would flow through
34 the membrane which contains zones of immobilised
35 capture antibodies. In the test zone, the 37KDa
36 fragment conjugate complexes would be captured by a

1 second antigen-specific antibody, forming a visible
2 line. If the 37KDa fragment is not present in the
3 urine, no visible line would form.

4

5 EGF-Receptor (Ab-4) is available from Oncogene Science,
6 Inc. as catalogue no. HCS16. There is no suggestion
7 that the antibody could be used to diagnose the
8 presence of the 37KDa EGFR fragment in urine or that
9 the presence of this fragment is indicative of bladder
10 or prostate cancer.

11

12 Other antibodies can be developed which are specific to
13 the 37KDa fragment. This may increase sensitivity of
14 the test.

15

16 A dip-stick test may be developed. This may require
17 using methods such as latex agglutination,
18 immunochromatography, ELISA and radioimmunoassay.

19

20 Bladder cancer prognosis has been correlated with a
21 number of factors, the single most important of which
22 is depth of invasion of the bladder wall
23 (Gospodarowicz, 1995); this is followed by grade of
24 tumour (Heney et al., 1983). Other less important
25 factors which influence patient outcome include tumour
26 size (Gospodarowicz, 1995), age of patient at diagnosis
27 (Fitzpatrick and Reda, 1986) and health status
28 (Thrasher et al, 1994). None of these factors can
29 predict prognosis in 100% of patients and so the 37KDa
30 fragment may have some use prognostically. The EGFR
31 fragment may be detected quantitatively using
32 densitometry following western blot analysis and used
33 to predict whether increased levels indicate a better
34 or worse prognosis. Other quantitative methods may be
35 developed to allow easier performance e.g. ELISA or
36 radioimmunoassay techniques.

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1 EGF and EGFR have been implicated in the pathogenesis
2 of solid tumours such as those of the breast. This
3 simple test developed for urine of patients with
4 suspected TCCB might also be used to identify the
5 diagnostic prognostic role of serum EGFR in other
6 tumour types.

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